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Lab on a fabric: Mass producible and low-cost fabric filters for the high-throughput viable isolation of circulating tumor cells

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ABSTRACT

Circulating tumor cells (CTCs) play an important role in estimating the presence and the metastatic relapse of tumor. Despite of their importance, isolation of viable CTCs is still struggling, since chemical or mechanical damages are unavoidable when separating less than 1000 of CTCs out of billions of other blood components. Furthermore, the current CTC isolation devices show low productivity, since they are produced after a series of complicated fabrication processes. Here, we present a low-cost and mass-producible fabric filters for the viable CTC isolation and the further molecular assay for profiling cancer-associated markers. The fabric filter, produced by polyester monofilament yarns, can be massively produced at extremely low-cost, by showing productivity of ~22 filters/s at ~59 filters/USD. By utilizing size-based sorting method, the fabric filter is capable to isolate both epithelial and mesenchymal CTCs, while slots with curved walls are beneficial for preventing the cell rupture by reducing 21.6% of mechanical stress compared to the conventional straight-walled slots. We applied our filter to 11 human blood samples and found that the number of CTCs was closely related to the expression level of Ki-67, which is highly overexpressed in proliferative tumors. The fabric filter might be an appropriate caner-screening tool in developing countries, where people suffer from insufficient healthcare services.

1. Introduction

Cancer is the second most fatal disease that leads to death (Jemal et al., 2011). An early diagnosis is highly necessary for the successful tumor removal, since the progression and metastasis is the major cause of death among cancer patients (Wicha and Hayes, 2011). Until now, imaging-based examinations using computed tomography (CT) or magnetic resonance imaging (MRI) are routinely conducted for the cancer diagnosis. However, the conventional CT and MRI require well-trained physicians with expensive equipment; thus the conventional cancer diagnosis methods might be inappropriate in developing countries. In spite of comparatively low incidence of tumor, the mortality in developing countries is similar to that of developed countries, due to the lack of healthcare services (Jemal et al., 2011). Another problem of the conventional imaging-based cancer diagnosis methods is that they are limited for detecting the tumor in a specific location. Invasive tissue biopsy is further necessary to characterize and to estimate the metastatic potential of tumor. Therefore, an alternative method which is not only cost-effective and less-invasive, but also capable of estimating the progressiveness of the tumor, is urgently required.

Circulating tumor cells (CTCs), originated from the primary tumor site, strongly involve in metastasis by flowing through the blood streams (den Toonder, 2011). CTC is accepted as a good predictor for estimating the state of tumor, since their number and the characteristics are highly associated with the tumor burden such as progression, proliferation, and metastatic potential (Spiliotaki et al., 2012; Tsai et al., 2016; Bluemke et al., 2009). Moreover, changes in the number of CTCs are known to represent the efficacy of anticancer drug treatments (de Bono et al., 2008). In spite of these remarkable findings and vast attentions, CTC isolation is still challenging and complicated due to their rarity and ambiguity. 0–1000 CTCs are found in the blood

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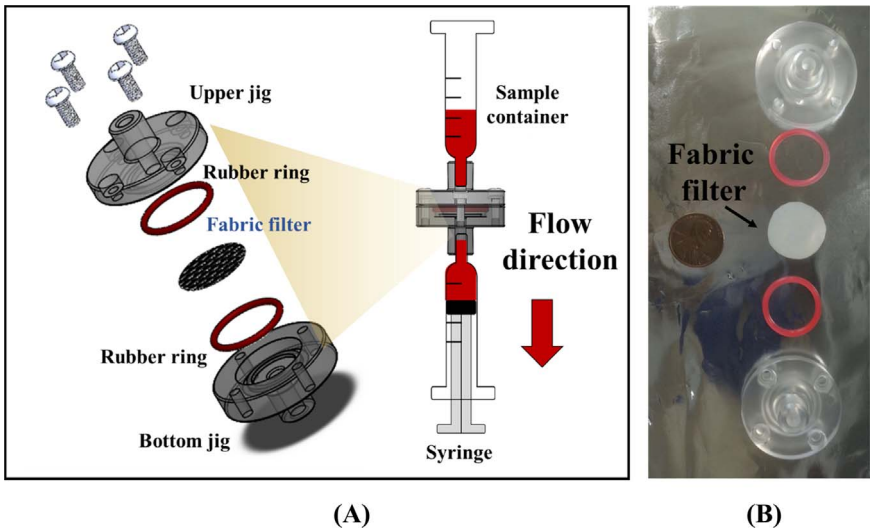

Fig. 1. The fabric filter based CTC isolation device: (A) schematic diagram; (B) manufactured fabric filter and its components.

samples of 1 mL obtained from cancer patients, while more than billions of other blood cells exist in the identical volume (Racila et al., 1998).

So far, the one and only CTC-based cancer diagnostic system, which have been approved by FDA, is CellSearch® system. CellSearch® system separates CTCs using antibodies against epithelial cell adhesion molecule (EpCAM) (Alix-Panabieres and Pantel, 2014). However, it is widely known that the expression level of EpCAM is downregulated when cells experience epithelial to mesenchymal transition (EMT), which is one of the most critical steps during metastasis (Karabacak et al., 2014). In this manner, CTC isolation methods based on anti-EpCAM antibodies might be unsuitable for finding CTCs with higher metastatic potentials. Other surface antibodies such as vimentin, plastin-3, and N-cadherin have been introduced for targeting these mesenchymal subtypes (Satelli and Li, 2011; Li et al., 2012; Sugimachi et al., 2014). However, suggested antibodies are restricted to the certain type of tumor or bind non-specifically to the untargeted leukocytes; thus resulting in low specificity and high false-negative ratio (Barriere et al., 2014; Lustberg et al., 2014). Furthermore, immuno-affinity based isolation methods (Nagrath et al., 2007; Yoon et al., 2013, 2014; Lu et al., 2013; Talasaz et al., 2009; Stott et al., 2010; Murlidhar et al., 2014) require long incubation time and complicated pre-processing steps, such as magnetic bead separation or erythrocyte removal, which make these methods inappropriate to be directly applied in clinics.

Alternative method for separating CTCs have been proposed recently, which utilizes differences in physical properties between CTCs and other blood components. These methods allow label-free CTC isolation, regardless of their surface antigen expressions. The remarkable differences, including size, deformability, density, and electrical properties, between CTCs and other blood components are used for separating CTCs (Gascoyne et al., 2009; Doh et al., 2012; Zheng et al., 2007, 2011; Kang et al., 2015; Adams et al., 2014; Fan et al., 2015; Tang et al., 2014; Yusa et al., 2014; Kim et al., 2016; Xu et al., 2010; Lim et al., 2012; Tan et al., 2009; Sollier et al., 2014). Among various methods, the size-based filtration has been widely explored, since it is one of the simplest methods that can acquire various CTC subtypes with minimal equipment settings. The current size-based CTC filtration devices are fabricated using various polymers, including, parylene (Zheng et al., 2011), SU8 (Kang et al., 2015; Adams et al., 2014), polydimethylsiloxane (PDMS) (Fan et al., 2015), polyethylene glycol diacrylate (PEGDA) (Tang et al., 2014), and palladium (Yusa et al., 2014). These devices have shown outstanding performance and have proven the clinical effectiveness. However, their low release efficiency (Kim et al., 2016) and low viability (Zheng et al., 2011) at the high flow rate conditions are problematic for the further downstream analysis. Moreover, the previous devices require a series of complicated fabrication processes using expensive materials, which is undesirable for the mass production (Green et al., 2016).

For the first time, we propose a fabric filter, made of polyester monofilament yarns, in order to isolate CTCs from human whole blood. The micro-sized slots, formed in between the neighboring warps and wefts, are used as size-based filtration slots for CTC isolation. Our filter can be massively produced at extremely low-cost compared to the previous CTC isolation devices, which require multiple fabrication steps using high-cost materials. In addition, curved walls inside the slots are expected to be beneficial for reducing the cell rupture during the isolation process. The curved walls which are naturally formed due to the circular cross-section of the monofilament can also enhance the retrieval efficiency by reducing the contact area between cells and the walls in the filter. The simple manufacturing method and the unique design of our fabric filter are expected to play an important role during high-throughput and viable CTC isolation. Our approach might provide a new cancer screening tool which can be extensively used in developing countries, where rapid and low-cost cancer diagnosis tests are strongly required.

2. Experimental section

2.1. Design of fabric filter

The fabric filters are composed of 20 denier polyester monofilament yarns, having diameter of 57.1 ± 3.6 μm. The density of warps and wefts were 592 ends and 200 picks per inch, respectively, which is denser than the conventional fabric sheets. The holes formed in between the neighboring warps and wefts are used as micro-sized slots for cancer cell separation. Figs. 1 and 2 show the schematic view and scanning electron microscope (SEM) images of the fabric filters. We produced two types of prototypes, by differentiating the twill structure. FF1 is composed of 2 by 2 twill structure (denim-like structure), having average width between neighboring warps and wefts of 7.6 ± 1.8 μm and 48.2 ± 2.9 μm, respectively. In case of FF2, which is produced in 3 by 1 twill structure, the distances between the neighboring warps and wefts were 12.1 ± 1.8 μm and 147.7 ± 2.1 μm. The fabric sheets were cut into circle with diameter of 20 mm. The fabric filter was joined by jigs, which can be connected to the commercial syringe, as shown in Fig. 1.